

Remarks/Arguments

Claims 1-25 and 27-46 are currently pending in the instant application. Claims 1-25 are under examination and claims 27-46 have been withdrawn from consideration. No claim amendments are submitted with the present Response.

Summary of the Invention

The invention disclosed and claimed in the present application is a method for purifying a polypeptide from a composition comprising the polypeptide and contaminants, which method comprises a number of sequential steps, namely (a) **loading** the composition onto an ion exchange resin with an equilibration buffer having a first salt concentration; (b) **washing** the ion exchange resin with a wash buffer until a predetermined protein concentration is measured in the flowthrough, wherein the salt concentration of the wash buffer increases from an initial, second salt concentration that is greater than the salt concentration of the equilibration buffer, to a final, third salt concentration; (c) passing a fixed volume of wash buffer at the final, third salt concentration over the cation exchange resin; and (d) **eluting** the polypeptide from the ion exchange resin with elution buffer that has a salt concentration that is greater than the final salt concentration of the wash buffer (see claim 1). Thus, the claims recite a sequence of steps, the relationship of salt concentrations employed in the various steps to each other, and the timing of performing washing step (b).

As described in the specification, the inventors found that the instantly claimed wash steps, performed in the order and using the parameters recited in the claims, were effective for polypeptide purification, particularly for resolving a desired polypeptide molecule from a contaminant differing only slightly in ionic charge. It is further recognized in ion exchange chromatography that the more similar the charge on the two molecules to be separated (polypeptide vs. contaminant), the more difficult it is to achieve a separation of the two molecules. The experimental examples in the patent application illustrate the use of the claimed methods to substantially reduce the amount of deamidated and other acidic variants in a purification of anti-HER2 antibody, which is a technically challenging separation owing to the very similar properties of the variants compared with the desired antibody polypeptide. In

Example 1, ion exchange chromatography was performed using a linear salt gradient (shown in Figure 2, as re-numbered), followed by a wash with 1 column volume (CV) of the wash buffer at the final salt concentration. In Example 2, ion exchange chromatography was performed using a multi-slope gradient (shown in Figure 3, as re-numbered), followed by a wash with fixed volumes of 0.4, 0.6 or 0.8CV.

In discussing the results from Example 2 it is stated on page 33 lines 5-8 that:

The results presented in Tables 4 and 5 show that it is possible to achieve consistent yield and acidic variant removal over a wide load range by using a multislope gradient wash. In addition, the results demonstrate that the trade off between yield and purity can be fine tuned as desired by adjusting the end-wash delay volume after an OD of 0.6 is achieved.

The "end-wash delay volume" referred to here is the wash using a volume of buffer at the final salt concentration or conductivity prior to elution, which links the wash step to any analytical measurements of the flowthrough. The effect of this wash provides an ability to increase purity of the eluted protein. (page 33 lines 9-13 of the instant specification).

The advantage that the instant method provides over the prior art is that, by using a three slope gradient wash, a greater range of resin load densities are possible while maintaining product quality and not sacrificing yield. The separation also becomes less sensitive to small variations in buffer composition.

Claims Rejections – 35 USC § 103

Claims 1-25 remain rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Genentech (Basey et al.; WO 99/57134) in view of Grandics et al. (US 5,571,720) and newly added reference Winge (US 2001/0034053 A1).

Basey et al. is cited for teaching protein purification by ion exchange chromatography. The Examiner acknowledges that Basey et al. do not teach an ion exchange chromatography system where a salt gradient is employed, and cites Grandics et al. and Winge et al. as allegedly filling this void. In particular, Grandics et al. is cited as allegedly teaching "varying salt gradients to elute the protein during the wash phase.". Winge is relied upon for allegedly

expressly teaching “increasing salt gradients/concentrations to purify the protein.” (Final Office Action, pages 2-4)

The rejection is respectfully traversed.

The combination of references cited does not teach or suggest all limitations of the pending claims.

To reject a claim based on obviousness, Office personnel must resolve the *Graham* factual inquiries which among other things articulate that the prior art must include each element claimed, although not necessarily in a single prior art reference, with the only difference between the claimed invention and the prior art being the lack of actual combination of the elements in a single prior art reference. MPEP 2143.

The combination of references cited does not teach or suggest all elements of the pending claims. In particular, the purification processes in the cited references do not disclose step (b) of claim 1, which requires

(b) washing the ion exchange resin with a wash buffer until a predetermined protein concentration is measured in the flowthrough, wherein the salt concentration of the wash buffer increases from an initial, second salt concentration that is greater than the salt concentration of the equilibration buffer, to a final, third salt concentration.

As discussed in the previous response, the cited prior art does not teach or suggest the methods of claim 1-25, which involve washing with an increasing salt concentration and then washing with a volume of wash buffer at the final salt concentration "until a predetermined protein concentration is measured in the flowthrough." Nor do the cited art references teach or suggest the multi-slope wash procedure of claims 12 and 13.

Basey et al.

Basey et al. teach a method for purifying a polypeptide from a composition comprising the following **washing steps**:

(b) washing the ion exchange material with an intermediate buffer at a second conductivity and/or pH so as to elute the contaminant from the ion exchange material; (c) washing the ion exchange material with a wash buffer which is at a third conductivity and/or pH, wherein the change in conductivity and/or pH from the intermediate buffer to the wash buffer is in an **opposite direction** to the change in conductivity and/or pH from the loading buffer to the intermediate buffer

(claim 1, emphasis added)

While the present application utilizes a progressive washing step with a continuous increase in the salt concentration, Basey et al. use a step-wise washing method based on **conductivity and/or pH** and a washing step that relies on a change in conductivity and/or pH that goes in the **opposite direction** of the previous wash step. The Examiner alleges that claim 11 of Basey et al. teach” a method of purifying a peptide by ion exchange chromatography, by specifically modifying the concentration of any salt employed.” (Page 3 of the instant Office Action). However, as acknowledge by the Examiner, “Basey et al. does not teach a salt gradient.” (Page 8 of the instant Final Office Action).

Grandics et al.

Grandics et al. teach the use of an integrated cell culture protein purification system using an ion exchange chromatography wherein

Unbound materials are washed with the binding salt solution and then product is recovered by applying a **low salt** buffer or a **reverse salt gradient to elute the protein of interest**.

(bottom col. 7 to top column 8; emphasis added)

In contrast to the presently claimed method, Grandics et al. do not teach applying a salt gradient of any kind, linear or non-linear, during the washing phase of the purification method. According to the Grandics method, the unbound materials are washed with the binding salt solution, which essentially means more of the same. It is only when the “product is recovered”, or during the elution phase, that Grandics contemplates applying a change in buffer conditions, i.e “low salt buffer or a reverse salt gradient.”

The Examiner alleges that Grandics et al. “teach varying salt gradients to elute the protein during the wash phase.” (Page 4 of the instant Final Office Action). The Examiner's assessment confuses a significant difference between the washing and eluting steps in chromatography. The claimed methods rely on washing with an increasing salt concentration to remove contaminants while the protein of interest remains bound to the column, until a predetermined protein concentration is measured in the flowthrough, at which time the protein of interest is eluted off of the column at a final salt concentration that is greater than the wash buffer. Thus, contrary to the Examiner's assertion, Grandics et al. do not provide the information missing from Basey et al. and therefore the combination of Grandics et al. with Basey et al. does not teach the invention claimed in the instant claims.

Winge et al.

Winge et al. only generally refer to the use of salt gradients with ion exchange chromatography and teach that “[e]lution of proteins can be carried out by stepwise higher salt concentrations or by linear or non-linear salt concentrations.” (paragraph [0043])

By contrast, the instantly claimed methods teach washing with an increasing salt concentration and then washing with a volume of wash buffer at the final salt concentration “until a predetermined protein concentration is measured in the flowthrough.”

The Examiner alleges that “Winge et al. expressly teach increasing salt gradients/concentrations to purify the protein.” (Page 3 of the instant Final Office Action). Once again, the Examiner misinterprets the teachings of Winge et al. and confuses the significant difference between the washing and eluting steps in chromatography. Winge et al. do not disclose or suggest the specific application of salt gradients in the washing step as claimed in the instant application, or the sequence of steps and process parameters recited in the pending claims. As discussed above for Grandics et al., Winge et al. do not teach applying a salt gradient of any kind, linear or non-linear, during the washing phase of the purification method. In all specific examples provided by Winge (Example 1 and 2, pages 5-7), a single salt concentration was used in the wash step and its duration was based on a predetermined volume of buffer being

passed through the column. By contrast, step (b) in claim 1 requires washing at increasing salt concentration until a predetermined protein concentration is measured in the flowthrough.

In summary, the 3 step washing phase of the currently claimed method wherein the salt concentration of the wash buffer increases from an initial to a second and third salt concentrations is not disclosed in any of the cited prior art references, when taken alone or in any combination. Instead, the prior art documents use a single salt concentration during the washing step. Contrary to the Examiner's assertions, the teachings within the cited references do not establish that washing using different salt gradients was known at the time of the invention and/or employed within the prior art. Neither are the methods claimed in the present application suggested by the cited combination of references, and thus represent a significant contribution to the art in view of the demonstrated unexpected effects achieved.

There is no motivation or suggestion to modify the cited references to reach the Applicants' invention.

The Examiner asserts that "varying the type of salt, amount (e.g. increasing OR decreasing concentration)" qualify as "routinely optimizable parameters." (Page 4 of the instant Final Office Action).

Applicants respectfully disagree. As described in the specification, the inventors found that the instantly claimed wash steps, performed in the order and using the parameters recited in the claims, were effective for polypeptide purification, particularly for resolving a desired polypeptide molecule from a contaminant differing only slightly in ionic charge.

As is recognized in ion exchange chromatography, the more similar the charge on the two molecules to be separated (polypeptide vs. contaminant), the more difficult it is to achieve a separation of the two molecules. The experimental examples in the patent application illustrate the use of the claimed methods to substantially reduce the amount of deamidated and other acidic variants in a purification of anti-HER2 antibody, which is a technically challenging separation owing to the very similar properties of the variants compared with the desired antibody polypeptide.

The advantage that the instant method provides over the prior art is that, by using a three slope gradient wash, a greater range of resin load densities are possible while maintaining product quality and not sacrificing yield. The separation also becomes less sensitive to small variations in buffer composition. Washing steps are often employed to remove a contaminant from the column prior to elution, however, the resolution of species that a gradient wash would technically provide would not appear valuable to one skilled in the art since those species will never be collected. Moreover, multi-slope gradients are very complicated to run in a manufacturing environment and it would strike the unfamiliar process developer as pointless to incorporate such a complicated operation into a wash phase that will be discarded. As discussed above, development of the multi-slope gradient used in the instant method was the solution to the problem of achieving a wide resin load density range while maintaining yield and purity.

The achievement of this improvement in the load density range is a significant improvement over the prior art. A complicated series of interactions occur within the ion exchange column during the wash step and acidic variants are essentially just teased off the resin charge groups. At low resin load densities there are a large number of unoccupied resin binding sites that the acidic variants can loosely re-associate with, which means that from one run to the next, as the amount of protein loaded on the column varies, it can take a longer amount of time or require greater conductivity to get the acidic variants off the column. The three slope gradient of the instant method is a novel and non-obvious solution to this problem. Together the multi-slope gradient wash followed by the step elution provides a method to consistently remove acidic variants with a robust, reproducible process that can be effectively automated and scaled up. The prior art taken alone or in combination would not lead a skilled artisan to apply the multi-slope gradient to the wash step as instantly claimed.

Applicants reiterate that the cited art does not offer suggestions or even recognize a need for modification of their respective methods. In the absence of any recognized failure or shortcomings of these cited methods, the Examiner has provided no motivation for a protein research scientist to pursue any modifications, let alone provide a reasoning for selecting the specifically claimed method of the instant application over the multitude of possible salt gradient combinations possible.

Specifically with regard to Grandics, Applicants submit that Grandics teaches the application of a low salt buffer or a reverse salt gradient. While this is common practice in purification methods using hydrophobic interaction columns, it is unsuitable for applications using ion exchange chromatography. In suggesting that one skilled in the art would combine the teachings of Basey et al., using ion exchange chromatography, and Grandics, using a method reversing the salt concentration from high to low during the washing and elution steps, the Examiner has erred in resolving the level of skill in the art. A person skilled in the art would not make this combination. Hence, there would be no motivation to apply the reverse salt gradient method of Grandics to achieve the instant method. Further, even if one were to consider the reverse salt gradient of Grandics, it would clearly result in an approach different from the instant method which comprises washing the ion exchange resin with a wash buffer, “wherein the salt concentration of the wash buffer increases from an initial, second salt concentration that is greater than the salt concentration of the equilibration buffer, to a final, third salt concentration, and eluting the polypeptide from the ion exchange resin with elution buffer that has a salt concentration that is greater than the final salt concentration of the wash buffer.” (Claim 1)

Finally, Applicants maintain that the Examiner’s conclusion of obviousness is based upon improper hindsight reasoning. As discussed above, the references cited by the Examiner do not teach or suggest protein purification via ion exchange chromatography employing washing steps with an increasing salt concentration to remove contaminants while the protein of interest remains bound to the column, until a predetermined protein concentration is measured in the flowthrough, at which time the protein of interest is eluted off of the column at a final salt concentration that is greater than the wash buffer. In addition, there is no motivation or suggestion to modify Basey et al., Grandics et al. or Winge et al. to reach the present invention, and no reasonable expectation that a combination of the reference with knowledge in the art would succeed in producing a protein purification method via ion exchange chromatography that achieves the claimed method. Thus, the only way the Examiner might have arrived at combining the cited references is using the disclosure of the present application to pick and choose selected portions of Basey et al., Grandics et al. and Winge et al., anion exchange chromatography, salt gradients and non-linear gradients respectively, in an attempt to recreate the claimed invention. Applicants respectfully submit that the Examiner’s conclusion is based upon an improper

hindsight reconstruction of the claimed invention, which, instead of looking at the prior art as a whole, picks and chooses teachings that appears to support a finding of obviousness, while completely disregarding others.

In summary, Applicants have shown that that the Examiner has not established a *prima facie* case of obviousness because: (1) the references cited by the Examiner do not teach or suggest protein purification via ion exchange chromatography employing different salt gradients during the washing phase, wherein the washing involves increasing salt concentration until a predetermined protein concentration is measured in the flowthrough. (2) there is no motivation or suggestion to modify the reference to reach the instant protein purification method because the references do not disclose protein purification via ion exchange chromatography employing different salt gradients during the washing phase, wherein the washing involves increasing salt concentration until a predetermined protein concentration is measured in the flowthrough. (3) there is no reasonable expectation of success that a combination of the references would succeed in producing a protein purification method utilizing ion exchange chromatography and increasing salt gradients due to the lack of disclosure of washing steps employing increasing salt gradients during the washing phase, wherein the washing involves increasing salt concentration until a predetermined protein concentration is measured in the flowthrough, and (4) the Examiner has based obviousness on improper hindsight. Accordingly, Applicants request that the rejection of Claims 1-25 under 35 U.S.C. § 103(a) as being allegedly unpatentable over Basey *et al.* in view of Grandics *et al.* and further in view of Winge *et al.* be withdrawn.

Conclusion

The present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

Please charge any fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 50-2387 (**Attorney Docket No.: 24126.064 (GNE-0113.US)**). Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: November 8, 2010

By Electronic Signature: /GINGER R. DREGER/
Ginger R. Dreger (Reg. No. 33,055)

ARNOLD & PORTER LLP
555 Twelfth Street, NW
Washington, D.C. 20004-1206
Telephone: +1 202.942.5000
Facsimile: +1 202.942.5999